REMARKS/ARGUMENTS

Claims 1-4, 7-11, 14-17 and 27-31 are pending in the application. Claims 3, 5-6, 10, 12-13 and 18-26 have been canceled without prejudice. Reconsideration of the rejection and allowance of claims 1-2, 4, 7-9, 11, 14-17 and 27-31 are respectfully requested.

35 U.S.C. §103

Claims 1-4, 7-11, 14-17 and 27-31 remain rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over Dubensky Jr. *et al.* (USPN 5,789,245, herein "Dubensky") in view of Yu *et al.* (Vaccine (1997) 15(12/13):1396-1404, herein "Yu"), both of record, and further in view of Harley *et al.* (*Clin. Micro. Reviews*, 2001, 14(4):909-932, herein "Harley").

The rejection is respectfully traversed as explained below.

The Examiner has asserted that Applicants have not demonstrated that Dubensky's method does not result in an equally pure product. The Examiner also asserts that it would have been obvious to use a filter pore size of less than 0.65 microns given that the diameter of an alphavirus is known and thus, the determination of the particular filter pore size ranges employed is within the skill of the ordinary worker and a part of the process of normal optimization.

As explained in previous responses, the methods of the present invention provide a surprisingly pure virus preparation through filtering. Notably, the reduction of any residual nucleic acid contamination is important in order to produce a pure virus product that is further applicable to large scale application. As explained previously, the reduction of DNA contamination is a critical step in vaccine production (see attachments provided with previous response).

Another major advantage of the invention is that the pure virus intermediate of the present invention is not substantially reduced during filtration. Applicants use a combination of filters that effectively purifies the product without resulting in substantial loss of the intermediate. As such, the Applicants have *surprisingly* found that the enveloped virus passes their filtering system without reduction of virus titer.

The Examiner's assertion that it would have been obvious to use a filter pore size of less than 0.65 microns is respectfully traversed. As has been pointed out previously, alpha virus particles are about 0.04 μm in diameter. Clearly, the size of the viral particles is much smaller than even the smallest pore size of the filters used in the claimed methods. The Examiner has provided no evidence why a second filter of a pore size of between 0.1 μm and 0.5 μm would be obvious in light of Dubensky and/or the alpha virus particle size of 0.04 μm.

Surprising Results

MPEP §2144.08 states that rebuttal evidence may include evidence that the claimed invention yields unexpectedly improved properties or properties not present in the prior art.[1]

Enclosed with this response is an unsigned declaration[2] from two of the inventors, Otfried Kistner and Manfred Reiter, providing evidence that the methods of the invention provided surprisingly better results than those achieved by the prior art method described by Dubensky *et al.* Applicants respectfully submit that these data demonstrate that the difference between levels of purity achieved in the prior art compared to the claimed methods are more than simple optimization as alleged by the Examiner.

As explained there, the declarants first cultured VERO cell infected with RRV, in a bioreactor and harvested the virus according to standard techniques. First, they followed Dubensky's teachings and passed part of the harvested virus through a 0.8/0.65 micron filter in order to clarify the crude RRV according to Dubensky's method (see column 120 in U.S. Patent No. 5,789,245). Second, they followed the teachings of the claimed method and passed part of the virus harvest after separation at ~9000g through a 1.2 micron filter and then through a 0.45 micron filter and finally through a 0.22 micron filter in order to clarify the crude RRV. They then assessed the purity of each virus intermediate through Vero-DNA, protein and TCID50 analysis.

The results showed that the RRV intermediate obtained with the claimed method has a DNA content of 11.8 ng (0.45μ filter) and 11.9 ng (0.22μ filter) per 10^7 TCID50 while the

[1] See MPEP §2144.08 (II) (B)
[2] A signed declaration will be forwarded to the Examiner when received by the undersigned.

RRV intermediate obtained with Dubensky's method has a DNA content of 95.7 ng DNA per 10^7 TCID50. They also compared the purity of the virus intermediates (obtained with each method) on a DNA to total protein basis and established that Dubensky's method produces an intermediate virus product of 1.62ng DNA per μg protein. In comparison, the claimed method produced a substantially higher purity of the intermediate with a DNA content of 0.23 ng per μg of protein (1.2/45μ filtration) and a DNA content of 0.08 ng per μg protein for the 1.2/0.45μ/0.22μ filtration. Both size exclusions, 0,2 and 0.45 were chosen according to the published pore size range of 0.1-0.5 micron. In addition, they filtered the 0.8/0.65 micron filtrate (intermediate according to Dubensky's method) with a 0.22micron filter. With this additional filtration step according to our method a significant decrease in DNA content to 63.4 ng/10^7 TCID50and an improved DNA/protein ratio 0.73ng per μg of protein could be achieved. For all experiments identical starting material with a TCID50 of 4.91×10^7 was used. The results are summarized in the tables below:

THE CLAIMED METHOD		
	DNA/Virus Titer [ngDNA/10^7($TCID_{50}$/ml)]	DNA/Protein [ngDNA/μgProtein]
Filtration: 1.2 μm/0.45 μm	11.8	0.23
Filtration: 1.2 μm/0.45/0.2 μm	11.9	0.08

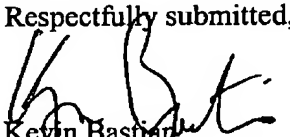
DUBENSKY'S METHOD		
	DNA/Virus Titer [ngDNA/10^7($TCID_{50}$/ml)]	DNA/Protein [ngDNA/μgProtein]
Filtration: 0.8 μm/0.65 μm	95.7	1.62
Filtration: 0.8 μm/0.65 μm/0.2μm	63.4	0.73

In conlcusion, the attached declaration demonstrates that the claimed methods provide dramatically purer virus preparations as compared to the cited prior art. In view of the surprising effectiveness of the claimed methods, the present rejection is improper and should be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at 415-576-0200.

Respectfully submitted,



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Attachments
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